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• REVIEW •

Primary adenocarcinomas of lower esophagus, esophagogastric junction and gastric cardia: in special reference to China

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Abstract

Gastric cardia adenocarcinoma (GCA) is an under-studied subject. The pathogenesis, molecular changes in the early stage of carcinogenesis and related risk factors have not been well characterized. There is evidence, however, that GCA differs from cancer of the rest of the stomach in terms of natural history and histopathogenesis. Adenocarcinomas of the lower esophagus, esophagogastric junction (EGJ) and gastric cardia have been given much attention because of their increasing incidences in the past decades, which is in striking contrast with the steady decrease in distal stomach adenocarcinoma. In China, epidemiologically, GCA shares very similar geographic distribution with esophageal squamous cell carcinoma (SCC), especially in Linzhou (formerly Linxian County), Henan Province, North China, the highest incidence area of esophageal SCC in the world. Historically, both GCA and SCC in these areas were referred to as esophageal cancer (EC) by the public because of the common syndrome of dysphagia. In Western countries, Barrett's esophagus is very common and has been considered as an important precancerous lesion of adenocarcinoma at EGJ. Because of the low incidence of Barrett's esophagus in China, it is unlikely to be an important factor in early stage of EGJ adenocarcinoma development. However, Z line up-growth into lower esophagus may be one of the characteristic changes in these areas in early stage of GCA development. Whether intestinal metaplasia (IM) is a premalignant lesion for GCA is still not clear. Higher frequency of IM observed at adjacent GCA tissues in Henan suggests the possibility of IM as a precancerous lesion for GCA in these areas. Molecular information on GCA, especially in early stage, is very limited. The accumulated data about the changes of tumor suppressor gene, such as p53 mutation, and ontogeny, such as C-erbB2, especially the similar alterations in GCA and SCC in the same patient, indicated that there might be some similar risk factors, such as nitrosamine, involved in both GCA and SCC in Henan population. The present observations also suggest that GCA should be considered as a distinct entity.

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INTRODUCTION

Adenocarcinomas of the gastric cardia are currently classified as gastric cancers[1, 2]. However, recent studies have suggested that these tumors have distinct epidemiological and biological characteristics[3, 4]. In the last two decades, the incidence of primary adenocarcinomas of the lower esophagus, esophagogastric junction (EGJ) and adenocarcinomas of the gastric cardia has increased dramatically in North America and Western European countries[3-6]. In contrast, the incidence of adenocarcinoma of the distal stomach has decreased steadily in recent years.

In China, adenocarcinomas of the gastric cardia have unique epidemiological features distinguishing these tumors from the adenocarcinomas of the distal stomach. Linzhou (formerly Linzhou County), the region with the highest incidence rate of esophageal cancer in the world, also has a high incidence of adenocarcinoma of the gastric cardia. However, the incidence of adenocarcinoma arising from the distal stomach is very low in this area. Therefore, previously, adenocarcinomas arising from the EGJ (gastric cardia) were referred to as esophageal cancers, because they shared similar clinical symptoms such as dysphagia. As most of the adenocarcinomas arising from the cardia are diagnosed at an advanced stage, it is very difficult to accurately define whether these tumors have a primary esophageal or gastric origin. Therefore, adenocarcinomas of the EGJ (gastric cardia) are now generally referred to as gastric cardia adenocarcinomas (GCA). With the exception of Barrett' s esophagus (a well documented premalignant lesion of esophageal adenocarcinoma in Caucasians)[7, 8], the role of other risk factors, such as *H. pylori* infection, diet, alcohol and tobacco exposure, still remains unclear.

Most studies of esophageal carcinoma in Linzhou have evaluated only esophageal squamous cell carcinoma (SCC). Few have studied adenocarcinoma (AC) of the gastric cardia, particularly as a separate pathological entity in China. In this review, recent epidemiological, histopathological and molecular biological advances were summarized to provide clues about the risk factors and the molecular carcinogenosis of adenocarcinomas of the lower esophagus, and EGJ (gastric cardia).

EPIDEMIOLOGY OF ADENOCARCINOMA OF THE GASTRIC CARDIA

GCA shares several common features with the adenocarcinoma arising from the distal esophagus: a lower mean age of

presentation, a higher incidence in the Caucasian population, a higher male to female ratio, and similar risk factors[4, 9]. Epidemiological studies have demonstrated that smoking, alcohol consumption, increased body mass index (reflecting obesity), and lower socioeconomic status are risk factors for adenocarcinomas of both the distal esophagus and gastric cardia[10-12]. In Linzhou, China, adenocarcinoma of the gastric cardia was previously classified as esophageal cancer by the local registry for the past several decades, primarily because of comparable clinical features and similarity in therapy. Reviewing hospital records of patients with dysphagia, our research group reported that while approximately 60 percent of patients were found to have a diagnosis of SCC, the remaining 40 percent were found to have adenocarcinomas of the lower esophagus or gastric cardia[13]. A case-control study performed on the patients with presumed distal esophageal or gastric cardia cancer in Linzhou found that one third of all these tumors was adenocarcinomas of the gastric cardia[14]. While there is considerable evidence implicating that dietary factors, including certain nutrient deficiencies and consumption of nitrosamine-contaminated food, and drinking water are associated with the development of SCC[15-18], it is not clear whether adenocarcinomas of the gastric cardia share the same risk factors in this high-incidence area in China.

DIAGNOSTIC CRITERIA FOR ADENOCARCINOMA OF THE GASTRIC CARDIA

It is generally difficult to distinguish where cardia adenocarcinomas originate, as most tumors present in advanced stage at diagnosis, often involving both esophagus and stomach. Imprecise clinical and pathological definitions of adenocarcinomas of the lower esophagus, EGJ or gastric cardia may be the reason underlying inconsistencies between different studies. An adenocarcinoma is considered to be of cardia origin when the epicenter of the tumor is at the esophagogastric junction, or within 2 cm distally[19]. Strict clinicopathologic criteria have been used also to define primary esophageal adenocarcinomas[20]. A new classification for adenocarcinomas of the esophagogastric junction was proposed at a recent conference of the International Gastric Cancer Association (IGCA) and the International Society for Diseases of the Esophagus (ISDE). Adenocarcinomas of the cardia has been defined as tumors that have their centres within 5 cm of the anatomic esophagogastric junction. Three distinct tumor entities (Type I, II and III) have been proposed[21].

RELAVANT PATHOLOGICAL CONDITIONS OF ADENOCARCINOMA OF THE GASRIC CARDIA

Barrett's esophagus

Barrett's esophagus is the condition in which columnar epithelium replaces the squamous epithelium that normally lines the distal esophagus[22]. The endoscopic appearance is characteristic, with a salmon-pink color and velvety texture. This upward extension may be circumferential, or limited to finger-like projections, or even islands of columnar epithelium that only involve part of the distal esophageal wall[23]. In healthy individuals, the squamo-columnar junction (SCJ) does not always correspond with the anatomic EGJ. The juxtaposition of pale squamous epithelium and reddish columnar epithelium forms a visible line called the Z line, or the SCJ. The EGJ is the most proximate part of gastric folds. The Z line and gastroesophageal junction often coincide. When the Z line is located above the EGJ, there is a columnarly lined segment of esophagus. Barrett's esophagus is diagnosed endoscopically if columnar mucosa extending greater than 3 cm above the EGJ, or by histologic finding of intestinal metaplasia (specialized mucosa) existing at any level of the tubular esophagus[22].

The pathogenesis of Barrett's esophagus is still unclear. Most Barrett's esophagus develops from approximately 10 % of persons who have chronic gastroesophageal reflux[24]. In addition to gastroesophageal reflux diseases (GERD), Barrett's esophagus is associated with several other esophageal disorders, such as hiatal hernia[25], and occasionally familial groupings are reported[26-28].

The significance of Barrett's esophagus is its association with the development of esophageal malignancy[29,30]. Patients with Barrett's esophagus have (by conservative estimates) a 30- to 40-fold higher risk of developing esophageal adenocarcinoma than those without[23]. Since the diagnostic criteria for Barrett's esophagus now include the histological finding of intestinal metaplasia, a columnar-lined esophagus less than 3 cm in length is now recognized as short-segment Barrett's esophagus (SSBE), in contrast to the traditional long-segment Barrett's esophagus (LSBE)[31]. The risk of malignancy in SSBE still needs to be determined[32].

In China, Barrett's esophagus used to be regarded as a rare condition, and consequently has not been studied in detail. However, based on an endoscopic survey performed by our research group in the high-risk population in Linzhou, Barrett's esophagus was detected with a frequency of 0.7 % (3/402); not as rare as it was considered previously[33]. It has been identified that there is an apparent change of Z line upgrowth among symptom free subjects in the high-incidence area for esophageal cancer in Henan Province. Z line upgrowth is related with epithelial cell hyperproliferation both in gastric cardia and lower segment of esophagus. These results suggest that Z line upgrowth may be one of the characteristic changes, or precancerous lesions related with EGJ carcinogenesis in the high-risk population for GCA in Henan[11]. Further characterizing Barrett's Esophagus, Z line upgrowth, and esophageal and gastric reflux esophagitis in this population may provide new insights into understanding of cardia carcinogenesis[34].

Intestinal metaplasia

Since the diagnostic criteria for Barrett's esophagus based on endoscopy are arbitrary, recent definitions of Barrett's esophagus have included the histologic finding of intestinal metaplasia, regardless of the length of the columnar-lined esophagus[35]. Histologically, three types of columnar epithelium have been observed in Barrett's esophagus: gastric fundic type, junctional type and specialized intestinal metaplasia (IM). Based on the mucin content of the columnar and goblet cells, intestinal metaplasia is histopathologically divided into three types: the complete form (Type I), in which the columnar cells are absorptive and contain neutral mucins, and the two incomplete forms, in which the columnar cells are partly secretory and contain acidic sialomucins (Type II) or sulphomucins (Type III)[36,37]. Type I is the predominant form of intestinal metaplasia found in the stomach, including both benign and malignant conditions. Type III is the least common form in the stomach, but the one most strongly associated with gastric cancer[38]. The incomplete form of intestinal metaplasia also termed specialized columnar epithelium (SCE), is the histological mark of Barrett's esophagus[39].

IM is also associated with GERD, and the frequency of IM is increased with the extention of columnar lining in the esophagus[40]. However, gastritis rather than GERD seems to be a risk factor of cardiac intestinal metaplasia[41]. While complete intestinal metaplasia is a manifestation of multifocal atrophic gastritis, the incomplete form may result from carditis and GERD[42].

It has been hypothesized that Barrett' s adenocarcinoma follows the metaplasia-dysplasia-adenocarcinoma sequence (MCS)[43]. Whether intestinal metaplasia is a premalignant lesion for adenocarcinoma of the gastric cardia is still not clear[44]. Several studies showed that dysplasia was infrequently observed in EGJ or gastric cardia with intestinal metaplasia[42, 44, 45]. These suggest that intestinal metaplasia at the EGJ or gastric cardia is not a high-risk precursor lesion for adenocarcinoma. However, progression from intestinal metaplasia at the EGJ or gastric cardia to dysplasia still requires further characterization[46].

Examination of the adjacent gastric cardia cancer tissue by our research group showed that IM was frequently observed in as high as 20 % of the specimens[47]. In contrast, only 0.7 % symptom-free subjects in Linzhou were identified with IM in gastric cardia biopsies[33]. Goblet cell was invariably identified on HE stained specimen. Half of the IM lesion was found to have dysplasia. These suggest that IM may be a precursor of gastric cardia adenocarcinoma in Henan, which is not consistent with that in Western countries. The reason is not clear. Standard histologic evaluation fails to reliably differentiate between IM in the esophagus and IM in native cardiac mucosa. Further characterization for subtypes of IM with biohistochemistry and immunohistochemistry will provide important evidence for elucidating the significance of IM in gastric cardia carcinogenesis.

Chronic carditis: H.pylori infection or gastroesophageal reflux disease

IM in the distal stomach is usually a consequence of chronic inflammation, mainly caused by *H.pylori* infection[48], while the intestinal metaplasia of Barrett' s esophagus has been demonstrated to be associated with GERD[49]. Furthermore, recent studies suggested that gastric infection with *H.pylori* might protect the esophagus from developing of reflux esophagitis and Barrett' s esophagus[50-54]. It is reasonable to hypothesize that intestinal metaplasia at the gastric cardia may develop as a consequence of chronic inflammation due to either GERD or infection. Several recent studies have focused on the relative contribution of *H. pylori* infection and GERD to gastric carditis, however, results have been inconsistent, to date[55-58]. One possible reason may be related to the use of different histopathologic criteria for identification of the gastric cardia. A study of 33 consecutive autopsies in children showed that the length of cardiac epithelium ranged from 1 to 4 mm, with a mean extent of 1.8 mm[59]. Ormsby expanded the study to 223 adult autopsies, finding that the mean extent of cardiac epithelium in patients aged <18, 19-50, and >50 years was 1.7, 2.6, and 3.3 mm, respectively[60]. Such a narrow range makes accurate sampling extremely difficult. Therefore, biopsies from the gastric cardia may have two origins: esophagus or stomach.

MOLECULAR BASIS FOR ADENOCARCINOMA OF GASTRIC CARDIA

The pathogenesis of carcinoma has been demonstrated to be a progressive, multi-step process, manifested as an uncontrolled cell cycle and abnormal proliferation. The molecular basis of human cancer has been investigated widely in the recent two decades, and has implicated deregulation of tumor suppressor genes, activation of oncogenes, and aberrant expression of growth factors. Different genetic alterations have been observed between the two different histologic sub-types of esophageal cancer. Meanwhile, molecular evidence is also accumulating to support the hypothesis that adenocarcinomas of the gastric cardia are distinct from adenocarcinomas of the esophagus and stomach.

Loss of heterozygosity (LOH)

LOH has been demonstrated to play an important role in tumorigenesis, and to be frequently associated with the loss of tumor suppressor gene function. LOH was initially observed at several tumor suppressor gene loci (17p, 13q, 5q, 18q and 9p for p53, Rb, APC, MSH2, DCC and p16, E-cadherin, 19p, respectively) in human esophageal and gastric cancer[61-67]. At present, LOH has been used to localize putative tumor suppressor genes. LOH has also been reported at 1p, 3p, 4q, 9q, 11p and 17q in esophageal adenocarcinomas[68, 69]. Allelic loss of the tumor suppressor gene p73 on 1p is frequently observed in neuroblastoma[70,71], and hMLH-1, a mismatch repair gene associated with hereditary non-polyposis colorectal cancer, is located on 3q21[72]. The tylosis esophageal cancer (TOC) gene, initially reported in families with autosomal dominant tylosis families who developed esophageal cancer, has been located on chromosome 17q25. Allelic loss at this region has been implicated in both sporadic SCC and Barrett' s adenocarcinoma[73]. BCRA1, associated with susceptibility to breast and ovarian cancer, is located on 17q[74]. Using comparative genomic hybridization (CGH), deletion of a specific region at 14q31-32.1 occurred significantly more frequently in Barrett' s adenocarcinomas in the distal esophagus than in gastric cardia cancers, suggesting genetic divergence in this group of closely related cancers[75]. Allelotype analysis performed on 38 gastric cardia adenocarcinomas even differentiated this allelic imbalance between the intestinal-type and diffuse-type adenocarcinomas, and a higher frequency of allelic imbalance on chromosome 16q was detected in the diffuse-type adenocarcinomas[76].

Dysfunction of tumor suppressor genes

Tumor suppressor gene p53 P53 is located on chromosome 17p13, encoding a phosphoprotein with a molecular weight of 53kd. Abnormalities in p53 gene function cause uncontrolled cell cycles and abnormal cell proliferation. P53 mutations are the most frequent genetic alterations found in human cancers[77]. Over 90 percent of p53 mutations are observed in exons 5-8, which is the highly conserved region for DNA binding. Comparison of p53 mutations in premalignant (basal cell hypertrophy, BCH; dysplasia, DYS; carcinoma *in situ*, CIS) esophageal tissues matched with corresponding invasive SCC tumor tissues, showed similar p53 mutations in DYS, CIS and tumors[78]. Similar studies with Barrett' s adenocarcinoma showed that p53 mutations in Barrett' s epithelia (metaplasia, non-dysplastic) did not necessarily correspond to the matched adenocarcinoma[20]. The high coincident alterations for P53 in SCC and GCA from the same patient indicate the possibility of similar molecular mechanisms, which provides important molecular basis and etiological clue for similar geographic distribution and risk factors in SCC and GCA[79,80]. These results suggest that some p53 mutations may have a selective tumorigenic advantage during tumor progression. And some findings suggest p53, bcl-2 and caspase-3 may play an important role in the induction of apoptosis in AGS cells[81]. In the course of the formation of gastric carcinoma, proliferation of gastric mucosa can be greatly increased by *H.pylori* infection, which can strengthen the expression of mutated p53 gene[82]. Less aggressive mutations may increase the genetic instability of Barrett' s mucosa, promoting abnormal cellular proliferation, but as they are incapable of transformation independently, additional molecular or epigenetic events are required for tumorigenesis.

About 90 percent of p53 mutations are located within the DNA binding domain (exons 5 to 8). They may occur either at the binding surface or at the hydrophobic core, disrupting the structure for DNA binding. P53 mutations do not distribute in a random manner through the whole encoding region. Several

"hot spot" mutations have been located such as codon 175, 176, 245, 248, 249, 273 and 282 in all the human cancers[83], implying that there may be a general pathway for the pathogenesis of tumors with a high percentage of p53 mutations. However, mutation sites also appear to be organ specific and cell-dependent. In esophageal adenocarcinomas, p53 mutations occur most frequently at codon 175 (9 %), 248 (16 %) and 273 (16 %), which are also hot spot mutations for all human cancers recorded, while mutations at codon 248 and 273 are much less frequently observed, even though the mutation at codon 175 accounts for 8 % in SCC. Relatively high frequencies of p53 mutations at codons 193, 194, 195 and 270 are unique in comparison to other human cancers[77].

P53 mutations may result from endogenous processes or exogenous carcinogens[84], and the spectrum of mutations may be indicative of specific carcinogenic mechanisms. Mutation profiles of p53 also help to identify the DNA damage arising from environmental carcinogens. Approximately 31 % of p53 mutations occur at the A:T base pairs in the SCC, which are usually caused by exogenous carcinogenic compounds. But in esophageal adenocarcinoma, p53 mutations have a very high frequency of transition at the CpG dinucleotides. This alteration is thought to result from spontaneous deamination of 5-methylcytosine, suggesting a defective DNA mismatch repair[77, 85]. Differences in p53 mutation spectra between SCC and esophageal adenocarcinoma are consistent with the results of epidemiological studies, suggesting that SCC and esophageal adenocarcinoma have different etiology.

Adenocarcinomas of gastric cardia share similar epidemiological and histological features to esophageal adenocarcinomas in North America and some European countries. Gleeson *et al*[86] compared the p53 abnormalities in adenocarcinoma of the distal esophagus and gastric cardia, and reported that p53 mutations were detected in 70 % and 63 % of adenocarcinoma of esophagus and gastric cardia, respectively. 85 % of the p53 mutations in esophageal adenocarcinoma occurred as G:C→A:T transitions, with 69 % at the CpG dinucleotides. Similar p53 mutations were observed in adenocarcinoma of the gastric cardia, in which 82 % were base transitions with 55 % occurring at the CpG dinucleotides. One study from Linzhou based on the p53 gene mutation analysis identified 6 mutations among 14 adenocarcinomas of the gastric cardia, 3 of which were G to T transversions, a mutation that is rarely observed in Barrett' s adenocarcinoma[87]. Further investigation and comparison of mutation analysis of p53 gene between SCC and adenocarcinoma of the gastric cardia in this population are needed so to provide more insights into the etiology of both SCC and adenocarcinomas of esophagus and the gastric cardia.

p53 gene family Recently, discovery of p53 homologues such as p73 and p63 suggests a more complex pathway. p73 and p63, similar to p53, may result in transactivation, DNA binding and oligomerization domains[88], capable of activating the transcription of p53-responsive genes and inducing apoptosis[89-91]. p73 is located on chromosome 1p36, a region that is frequently deleted in neuroblastoma[73, 74], but unlike p53, p73 mutations have been detected infrequently in other types of tumor, including esophageal and gastric carcinoma[92-100]. Silencing of p73 gene due to hypermethylation at its promoter region was observed in a subset of lymphoblastic leukaemia and Burkett' s lymphoma[101, 102], but increased level of mRNA was more commonly observed in other tumors[95, 103-105]. p73 is characterized by loss of imprinting (LOI), resulting in monoallelic expression in normal tissues. It has been observed that p73 is overexpressed in several tumors, which is thought to be due to the switching from monoallellic to biallelic expression[93, 95]. Meanwhile, p73 isoforms, caused by alternative splicing, have been observed in rare types of brain tumors, suggesting tissue-specificity in the regulation of p73 gene transcription[106]. However, the precise function of p73 isoforms in tumor development is still unclear. Specific mutations of p73 or p63 causing amino acid substitutions are not identified. Neither p53, p73 nor p63 is related to prognosis. p73 and p63 have rarely been found to be mutated in gastric carcinomas, but both proteins are expressed in only a subset of tumors. The status of these p53 homologues is discordant among all patients with multiple simultaneous gastric carcinomas. The increased expression of p63 (TAp63 and black triangleNp63) in less well-differentiated gastric carcinomas may indicate that p63 can act to promote neoplastic growth in the gastric epithelium[107]. In 15 SCC samples from Henan, no p73 mutations were found in exons 4-7, but a high frequency of LOI and LOH was observed in these samples. The SCC samples with p53 defects were significantly correlated with those, which had elevated expression of p73. These results suggest that increased expression of p73, including that by LOI, could be a partial compensatory mechanism for defective p53[100].

The p63 gene also encodes multiple isoforms through alternative splicing with different abilities to transactivate p53-responsive genes. However, p63 is more closely related to p73 than to p53, and p63 mutations have been rarely observed in human tumors[108, 109]. The predominant isoform of p63, lacking in an acidic N-terminus corresponding to the transactivation domain of p53, has been detected in many kinds of epithelial tissue. This truncated protein may act as a dominant-negative agents toward transactivation by p53[110]. Up to date, p63 has not been studied in esophageal adenocarcinoma or adenocarcinomas of the cardia.

Rb gene One study compared LOH with the expression of Rb gene in SCC, finding that 90 % of the tumor samples containing LOH showed low or no Rb expression, while only 20 % of those without LOH had altered expression of Rb, suggesting LOH is the principal molecular alteration of Rb in SCC[111]. Recent studies of SCC cell lines demonstrated that the hypophosphorylated Rb protein was also associated with G2 arrest[112]. Besides inhibition of the cell cycle progression, Rb protein appears to have other distinct mechanisms to suppress cellular proliferation[113]. In the gastric carcinogenesis, *H pylori* might cause severe imbalance of proliferation and apoptosis in the precancerous lesions (IMIII and DysIII) first, leading to p53-Rb tumor-suppressor system mutation and telomerase reactivation, and finally causing gastric cancer[114]. Wang *et al*[115] suggested the alteration of Rb protein might play a role in the early stages of gastric cardia carcinogenesis.

p16INK4a, p15INK4b and p14ARF LOH of p16INK4a has been detected with high frequencies in both SCC (65 %) and esophageal adenocarcinoma (69 %)[116]. Several studies have demonstrated that hypermethylation of the promoter region of p16INK4a is the main mechanism causing gene silencing rather than point mutation, which rarely occurrs in esophageal carcinomas[116-119]. Distinct from p16INK4a, p15INK4b, which inhibits the cell growth in response to extracellular stimuli such as TGF-β[120], is more frequently deleted, at a frequency of 40 %[118]. Though p16INK4a regulates cell cycle through inhibiting the phosphorylation of Rb protein, p14ARF is closely associated with p53 by attenuating mdm2-mediated degradation of p53[121,122]. Similar to p15INK4b, p14ARF is also deleted frequently in SCC, losing its function to protect p53 from ubiqitin-dependent degradation[123].

Abnormalities of oncogenes Point mutation, amplification, rearrangement and overexpression are the most frequent mechanisms for oncogene activation. Amplification of cyclinD1, HER-2/neu(c-erbB2), c-myc, c-ras, Int-2/hst-1 and c-erbB (EGFR) has been observed in gastric and esophageal carcinomas[124-130]. The amplification of c-erbB2 was followed

by overexpression in the same gastric adenocarcinoma tissue[125,129]. No amplification of c-erbB2 was detected in SCC. Amplification of oncogenes that encode growth factor receptors is more commonly found in adenocarcinomas. Int-2 and hst-1, encoding FGF-3 and FGR-4 respectively, are located at chromosome 11q13 with the oncogene cyclinD1. Coamplification of int-2 and hst-1 was observed at a frequency of 28-47 % in primary esophageal carcinomas and much higher in metastatic tumors, suggesting an association with tumor progression and distal metastasis[131, 132]. CyclinD1, a cell cycle protein, facilitates cell cycle progression from G1 to S phase through combining with CDK4. Both the amplification and overexpression of cyclinD1 were detected in esophageal carcinomas[133, 134]. Abnormal expression of cyclin E and p27 may be one of the important molecular changes in the early stage of esophageal carcinogenesis, and the high-expression of cyclin E and low-expression of p27 may be one of the mechanisms driving the mild lesion towards carcinogenesis[135]. In contrast to other gastrointestinal tumors, the ras oncogene is rarely mutated in human esophageal adenocarcinoma[127]. However, overexpression of ras and ras-regulated genes (osteopontin, cathepsin L) has been reported in 58 % of primary esophageal adenocarcinomas[136].

DIFFERENCES BETWEEN ADENOCARCINOMAS OF LOWER ESOPHAGUS, GASTRIC CARDIA AND SUBCARDIAC STOMACH

Though adenocarcinomas of the lower esophagus and gastric cardia share similar epidemiological characteristics, new clinicopathologic classifications have established criteria to differentiate these two entities. Taniere[137] compared molecular markers in esophageal adenocarcinomas with those in adenocarcinomas of the gastric cardia. The male to female ratio and p53 mutation frequency were higher in esophageal adenocarcinomas, while mdm2 amplification was more frequent in the adenocarcinomas of the gastric cardia. Patterns of cytokeratin immunostaining were also different between these two tumors. Flejou[138] performed a study to compare p53 protein expression immunohistochemically between esophageal and gastric carcinomas, and reported a higher prevalence of p53 protein overexpression was found in esophageal and cardiac adenocarcinomas compared with gastric antral adenocarcinoma. This was confirmed by an additional study, which reported p53 mutation rates in adenocarcinomas of distal stomach were significantly lower than those in adenocarcinomas of esophagus and gastric cardia[139]. These results suggest that adenocarcinoma of the gastric cardia may be a distinct entity from adenocarcinoma of the distal stomach.

Comparison between gastric cardia adenocarcinoma and esophageal squamous cell carcinoma, with special reference to Linzhou

Because of the highly concurrent incidence of gastric cardia adenocarcinoma and esophageal squamous cell carcinoma in Linzhou[140], it is highly desirable to characterize the molecular differences between these tumors of different histological types to explore the possible clues related with etiological risk factors. However, useful information concerning this topic is very limited. In Linzhou, it is not uncommon clinically to identify patients with both gastric cardia adenocarcinoma and esophageal squamous cell carcinoma[141], which is the most common pattern of multiple primary malignant neoplasm (MPN) with an incidence of 0.4-2.5 %[142]. Recent studies from Wang's laboratory showed that there was a highly consistent positive immunostaining rate for p53 in SCC and GCA from the same patient (60 %, 12/25 *vs* 40 %, 10/25)[79] similar results were observed for PCNA. p53 mutation analysis in patients with either SCC or GCA from Linzhou indicated that G:C to A:T transition was the most frequent mutation pattern both in SCC and GCA. The consistency of P53 gene mutation was as high as 64 %[143]. G to A mutation pattern may be resulted from DNA methylation induced by nitrosamine[141]. These results are of important value in explaining the similar geographic distribution of SCC and GCA in this area. Protein file analysis also showed similar expression pattern in SCC and GCA, such as PTEN[144], c-erbB-2 and c-myc[145], MUC1[146], CYP1A1 and 2E1[147], MUC3[148], mEH[149], GSTM1, GSTT1 and GSTP[150], EGFR[151], etc from the samples in Linzhou.

CONCLUSION

In summary, the incidence of adenocarcinomas arising from the distal esophagus, EGJ and gastric cardia is increasing at a dramatic rate in North America and Europe. It is suggested that adenocarcinomas of the esophagus develop in a metaplasia-dysplasia-carcinoma sequence. Whether adenocarcinomas of the gastric cardia follow the same pattern needs further study. Although both esophageal squamous cell carcinomas and adenocarcinomas of the gastric cardia have a high incidence in the same high-risk population in Linzhou, little information is available on the molecular and etiological differences between these two tumor types. Metaplasia is a frequent finding in the gastric cardia, but its significance in the development of adenocarcinoma of the gastric cardia is not clear. *H.pylori* infection, a main factor causing atrophy of the mucosa of distal stomach, also leads to chronic inflammation of the gastric cardia. Though *H.pylori* has been classified as a Type I carcinogen, the role it plays in gastric cardia carcinogenesis remains unclear. To study adenocarcinoma of the gastric cardia in China, researchers are beginning to evaluate epidemiological, histopathologic and molecular characteristics of the tumor.

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